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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Removal of Chemical Oxygen Demand, Total Organic Carbon and Color from Textile Industry Wastewater by Using *Phanerochaete chrysosporium* (ME446)

Numan YILDIRIM[1] Gökhan Önder ERGÜVEN[2]* Aytekin ÇELİK[3]

[1]*Department of Plant and Animal Production, Tunceli Vocational School, Munzur University, TR62000 Tunceli, Turkey*
[2]*Munzur University, Faculty of Economics and Administrative Sciences, Department of Political Science and Public Administration, Department of Urbanization and Environmental Issues, TR62100, Tunceli, Turkey*
[3]*Firat University, Faculty of Engineering, Department of Environmental Engineering, 23720, Elazig, Turkey*

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*: https://orcid.org/0000-0003-1573-080X
: https://orcid.org/0000-0003-1109-8106
: https://orcid.org/0000-0003-4234-1797

***Corresponding author's:**
Gökhan Önder ERGÜVEN
Munzur University, Faculty of Economics and Administrative Sciences, Department of Political Science and Public Administration, Department of Urbanization and Environmental Issues, TR62100, Tunceli, Turkey
✉: goerguven@munzur.edu.tr

Abstract: Wastewater discharge from textile industries concern environmental risks. Superiority of microbial methods over high cost combined methods includes conversion of persistent organic materials to non-toxic last materials, sustainability, low cost, and comfortable. Textile wastewaters can have opposite effects on the quality of water in total organic carbon (TOC) and chemical oxygen demand (COD). In this study, Biotreatment of textile wastewater from the dyeing process using white-rot fungus *Phanerochaete chrysosporium* (P.C) was investigated in agitated and static culture conditions to solve these problems. The treatment mediums containing distilled water in 1:10 ratio of wastewater were compared for treatment efficiency of P.C. Especially in agitated conditions at 27°C and 150 rpm, it was achieved a successful treatment results. Under these conditions, a 48h long treatment reduced by 91,46 % of the original COD (from 1484 ppm) and by 94,92% the TOC (initial was 723.66 ppm). Moreover, treatment reduced color by 86,28 % from 3.550 A_{540} to 0.487 A_{540} at the end of the study. The decolorization properties of P.C obtained high performance and we determined P.C showed up to effective removal efficiency for COD and TOC within 48 hours. As a result, it was determined that, these fungus pellets of P.C reached the high bioremediation efficiency and can be a useful tool for bioremediation of textile dye wastewater within a short time period.

Keywords: Bioremediation, COD, *P. chrysosporium*, textile wastewater, TOC.

Tekstil Endüstri Atıksuyundan *Phanerochaete chrysosporium* ile KOİ, TOK ve Renk Giderimi

***Sorumlu yazar:**
Gökhan Önder ERGÜVEN
Munzur Üniversitesi, İktisadi ve İdari Bilimler Fakültesi, Siyaset Bilimi ve Kamu Yönetimi Bölümü, Şehirleşme ve Çevre Sorunları Bölümü, TR62100, Tunceli, Türkiye
✉: goerguven@munzur.edu.tr

Öz: Tekstil endüstrilerden kaynaklı atıksu deşarjı çevresel riskler teşkil eder. Mikrobiyal metodların diğer yüksek maliyetli kombine metodlara nazaran üstünlüğü kalıcı organic materyallerin toksik olmayan nihai ürünlere dönüşmesi, sürekliliği, düşük maliyeti ve uygulanırlığıdır. Tekstil atıksuları su kalitesine toplam organic karbon (TOK) ve kimyasal oksijen ihtiyacı (KOİ) bakımından olumsuz etki yapar. Bu çalışmada boyar madde prosesinden gelen tekstil atıksuyunun beyaz kök mantarı *Phanerochaete chrysosporium* (P.C) ile biyoarıtımı çalkalamalı ve statik kültür koşullarında bu problemleri çözmek için araştırılmıştır. 1:10 oranında distile su içeren atıksudaki arıtma ortamları P.C' nin arıtma verimiyle kıyaslanmıştır. Özellikle çalkalamalı kültür koşullarında 27°C'da 150 rpm'de oldukça uygun bir arıtma sonucu elde edilmiştir. Bu koşullar altında, 48 saatte arıtım orjinal KOİ'de %91,46'ya (1484 ppm'den) ve TOK bakımından ise %94,92 (giriş değeri 723.66 ppm'dir). Bunlara ilave olarak çalışma sonunda renkdeki giderim 3,550 A_{540}'den 0.487 A_{540}'a %86.28'in altına düşmüştür. P.C'nin dekolorizasyon özelliği yüksek bir performans elde etmiştir ve P.C; KOİ ve TOK'da 48 saatte etkili bir giderim oranı göstermiştir. Sonuç olarak, P.C yüksek bir biyoremediasyon oranına ulaştığı ve tekstil boyar madde atıksularının biyoremediasyonunda kısa zaman periyodunda kullanılabilir olduğu tespit edilmiştir.

Anahtar kelimeler: Biyoemediasyon, KOİ, *P. chrysosporium*, Tekstil atıksuyu, TOK.

INTRODUCTION

The textile industries consist of hundreds of different type of dyes that can be stay in the presence of some chemical substances, light vulnerability and also biological degradation. In the world, 10.000 synthetic dyes being used. Since their essential refractoriness, 90% of reactive textile industry dyes stay in the sewer plant systems and at the end, dismissed into receiving environments. Every year, around 30 x 10^4 tons of industrial textile dyes are dismissed into the wastewater channels (Ogugbue et al., 2011). These wastewaters have opposite effects on the receiving bodies, not only by flogging waters but also by mutagenic capacity and target toxicity (Singh et al., 2015). Wastewater discharge from textile and other common industries produces environmental risks (Saratale et al., 2011). In textile wastewater discharging, the common environmental problem is dyes (Yildirim et al., 2018).

Although a too many textile industries are linked to effluent treatment units, the partially treated wastes is discharged into the receiving environments like seas, rivers, lakes or streams. When discharge levels and waste composition are regarded, the textile facility is the most polluting sector in the world (Miled and Ladhari, 2020).

Environmental problems are related to the associated colour, high COD values, low biodegradability, fluctuating pH and high temperature of the wastewater (Al-Kdasi et al., 2004). Coagulation-flocculation followed by sedimentation or adsorption is generally used, but is not encouraging due to the secondary pollution arising from the residual sludge. Aerobic biological treatment methods are the most prefered methods in the textile wastewater plants but have low efficiency with synthetic dye waters, which represent more than 80% of the annual consumption in theses industries (Tounés et al., 2018). The existent chemical, physical and photochemical approach for the removal of such dyeing wastewaters have some opposite properties like cost, associated functional and operational problems, and large amounts of sludge that consists hazardous materials (Srinivasan et al., 2010). In order to discharge effluents that meet legislative bodies, innovative processes using advanced techniques have been tested and applied in several ways (Miled et al., 2015). Most of these methods can only transfer the effluents from one phase to the other leaving the problem fundamentally unsolved. Additionally, such storage systems do not include attractive possibilities of recycling because of storage is unsatisfactory. Wastewater treatment from reactive and direct dyes involves some complications due to the high salinity, which makes reprocess of treated wastewaters impossible for irrigation activities (Khatri et al., 2015). Environment friendly technologies have also been recommended in order to reduce the impact of these kind of materials generated from industrial plants (Kumar et al., 2018). To handle these problems, over the last years, biological methods such as bioremediation and biosorption by both living and dead microorganisms in aerobic, anaerobic or facultative treatment methods with microorganisms have been preferred (Robinson et al., 2001). A wide range of combined methods have been used to manage textile dyes (Saratele et al., 2011). Although the chemical and physical processes are seemed to be practical for removal of dyes, they have also constitutional difficulties such as high operative cost, and toxic last products and exhaustive energy uptake (Arora et al., 2017, Yonten et al., 2016).

Fungi and bacteria have also been shown to efficaciously decolorize and biodegrade textile wastewater dyes (Asgher et al., 2013, Hossain et al., 2016, Urrea et al., 2020). The role of fungus has been recently chosen and shown to be related to the biodegradation of dyes (Yildirim et al., 2018, Yonten et al., 2016). There is still limited information about the influence of decolorization processes on water ecosystems (Przystas et al., 2015). White rot fungi (*P. chrysosporium*, *Pleurotus* sp, *Trametes* sp, *Ganoderma* sp) are among the most suitable fungi for the removal of synthetic reactive dyes. In nature, these fungi colonize wood and other lignocellulosic substrates and knowledge of their capacity to remove recalcitrant dyes from wastewater. Different extracellular enzymes of the fungi are able to oxidize dye compounds (Novotny et al., 2004).

This study reveals out the common results obtained during the laboratory scale studies on the reduction of TOC, COD, and color with a newly isolated white rot fungi *P. chrysosporium* as an inexpensive alternative process for degradation of textile dye bath wastewater existing from the dyeing process. The study depends on find out the efficiency of the *P. chyrsosporium* for bioremediation of textile wastewater.

MATERIAL AND METHOD

Wastewater samples: The red-colored textile wastewater was obtained from Tekirdag province of Turkey. This dye bath wastewater from the dyeing process was bottled in a plastic container and kept at 4^{o}C. The physicochemical characteristics of used wastewater are demonstrated in Table 1. The wastewater was sterilized by an autoclave at 121^0 and 1.5 atm about 15 minutes before used in bioremediation assays.

Table 1. Characteristics of textile wastewater.

Parameters	Values
Absorbance, A_{540}	3.550
COD (ppm)	1484
TOC (ppm)	723.66
pH	10.2

Fungus and pellet preparation: *Phanerochaete chrysosporium* (ME446) were taken from Hacettepe University, Department of Biology and was stored at 4°C, after subculturing at 27°C every two-three weeks on sabouraud dextrose agar (SDA). The fungus was cultured at 27°C on SDA plates according to (Zelles at al., 1991). After 4 days of incubation, mycelial plugs (5 mm diameter) from the peripheral region of an actively growing culture were used as inoculum. These mycelial plugs were transferred into 100 mL Sabouraud dextrose broth (SDB) in a 250-mL flask. Incubations were performed in a shaking incubator (150 rpm) at 27°C for 4 days. After incubation step, the culture was homogenized and used for the inoculation for pellet preparation. Fungal pellets were gathered by filtration from culture media and wet pellets (500 mg) were used in the decolorization for additional experiments.

Decolorization assays: About 500 mg wet fungal pellets (5000ppm) were transferred into 100 mL the sterilized wastewater in 250 mL flasks. The agitation rate and temperature were 150 rpm and 27 °C, respectively in orbital shaked incubator for 24, and 48 h. For decolorization, COD and TOC experiments, all studies were performed with 1/1 (crude, undiluted) and 1/10 (diluted with deionized water) samples of textile wastewater. This dilution rate was for reduce the inhibation effect of wastewater on fungus. At last, the bioremediation efficiency increased.

Decolorization of wastewater was measured in filtrates (three replicate flasks) after eliminating the pellets, and monitored spectrophotometrically at the maximum wavelength of 540 nm absorbance (Harry et al., 1991). The systems without the fungus served as abiotic controls.

$$\text{Decolorization (\%)} = \frac{\text{Ax} - \text{Ay}}{\text{Ax}} \text{ x } 100$$

Where, Ax = Absorbance of the blank (wastewater),
Ay = Absorbance of the treated dyes solution after incubation.

Physico-chemical analysis: Color experiments were performed with UV-Visible spectrophotometers (Shimadzu H524148) at Abs_{540}nm. The COD experiments were performed with HACH DRB 200 thermoreactor (heating phase to 148^0C) and HACH DR 890 colorimeter (reading phase). Hach COD kits that have measurement capacity in the range of 0-1500 mg O_2/dm^3 (Cat number: 23459-52) used by the line of Standard Method 5220C closed reflux method. For inhibiting the microbial activity, only one drop of 0.1N H_2SO_4 added to the samples before transfered to COD or TOC device. For this process, 2.5 ml of samples added to the COD kits that contains 3.5 ml $AgNO_3$ and 1.5 ml of Cr_20_7. After that, the COD kits were taken to the HACH DRB200 thermoreactodr at 150 C^0 for two hours. After this time finished, COD kits were taken to the room temperature for cooling. The cooled samples were put on a HACH DR 890 colorimeter and COD values were readed at 540 nm. Abs and level of decreasing followed. TOC determinations were performed with Standard Method 5310A (burning at a high temperature) with SHIMADZU TOC-V. TOC is universally measured by oxidizing the organic compounds present to forms which can be quantified. High-temperature combustion at 1200°C in an oxygen-rich atmosphere. The CO_2 produced is passed through scrubber tubes to remove interferences and measured by non-dispersive infrared absorption (NDIR). According to this method, samples were filled to 5 ml tubes and settled to TOC analyzer device in Laboratory of Firat University Environmental Engineering Department and decreasing of the TOC parameter followed like COD assays. All of the experiments were performed at room temperature (25^0C) according to the methods described in (APHA, AWWA, 2005).

Statistical analysis: SPSS version PASW Statistics 18 was performed for analysis of all data (SPSSInc., Chicago, IL, USA). ANOWA was carried out to analysis of data and when $p<0.05$, results was considered significantly different. Differences between means were analyzed using Duncan's multiple range test for post hoc multiple comparisons. Values were presented as the mean ± standard error (Anova, 2017)

RESULTS AND DISCUSSION

The live pellets of P.C performed a rapid wastewater decolorization activity for decolorizatin, COD and TOC parameters in 48 hours. 59,16 and 86,28% decolorization was observed after 24h and 48h incubations in agitated conditions with crude wastewater, respectively. In static conditions, the highest decolorization activity was observed as 42,49 and 62.34 % after 24h and 48h incubations with crude wastewater, respectively (Fig. 1).

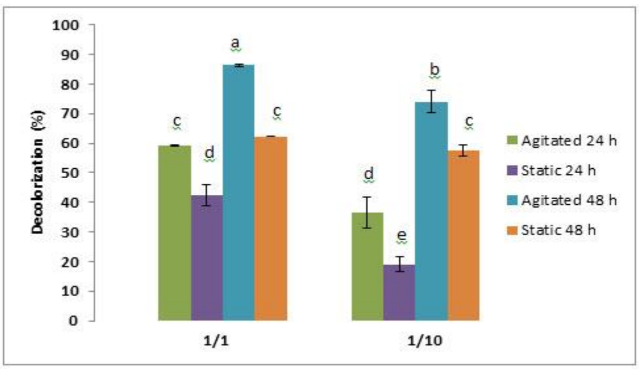

Figure 1. Reduction of decolorization in media with agitated and static conditions at the end of 24^{th} and 48^{th} hours. Different letters on the bar (a,b,c,d,e) show statistical differences of Duncan's multiple range test among all groups ($p<0.05$).

The highest COD reduction was achieved by pellets as 91.46 % in the agitated medium with crude wastewater after 48h incubation. The lowest reduction efficiency was noted as 58,19% in static medium with crude wastewater after 24h incubation. Generally, agitated mediums with crude wastewater were showed high COD reduction efficiencies than static mediums with 1:10 diluted wastewater (Fig. 2

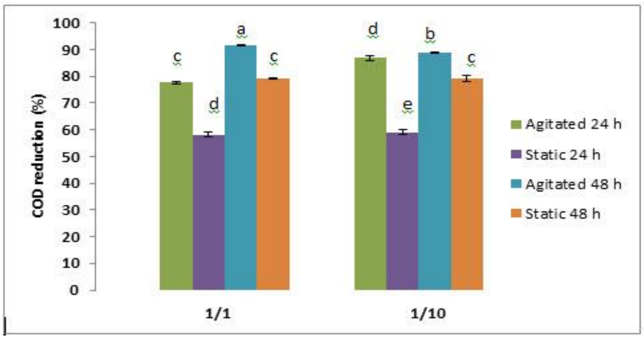

Figure 2. Reduction of COD in media with agitated and static conditions at the end of 24th and 48th hours. Different letters on the bar (a,b,c,d,e) show statistical differences of Duncan's multiple range test among all groups ($p<0.05$).

According to the results, best removal performance occurred for TOC parameter in agitated conditions as 94.63 and 94,92% at the end of the 24h and 48h respectively in media with 1:10 diluted wastewater (Fig. 3).

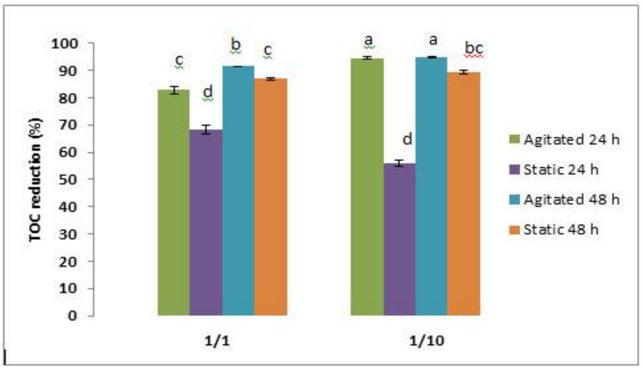

Figure 3. Reduction of TOC in media with agitated and static conditions at the end of 24th and 48th hours. Different letters on the bar (a,b,c,d,bc) show statistical differences of Duncan's multiple range test among all groups ($p<0.05$).

While the highest color and COD removal was observed in crude wastewater medium, the highest TOC reduction was obtained in the medium with 1:10 diluted medium. For all three parameters (Color, COD, and TOC) the highest removal values were obtained under agitated conditions.

The changing in the color removal of other reactive dyes might be attributable to the morphological difference of the textile industry wastewaters and used microorganisms (Kalyani et al., 2008). Most dyes have high levels of COD in their compositions and can have opposite effects on the quality of water in TOC and COD parameter (Mahmoud et al., 2017).

The obtained results gained from the treated wastewaters showed, *P. chrysosporium* could effectively decolorize the textile wastewater. Color removal efficiency was obtained approximately as 87% when wastewater was not diluted (crude) in agitated medium. Our results are more standardized to the results obtained recent studies on decolorization of textile wastewater by *P. chrysosporium.* The effect of concentration on decolorization of textile wastewater was performed, Our results revealed that the decolorization rates were high at 1:10 concentration of the wastewater. This may be due to the fungal growt was inhibited by crude wastewater (1/1). The similar results werea already reported by Novotny et al. (2004). In all the cases color removal decreased by decreasing the initial concentration of the wastewater. There were similar observations on decolorization of textile wastewater by *P. chrysosporium.* Decolorization efficiencies were found to be higher in agitated culture conditions than static culture conditions. Homogenous physical and chemical environments caused by agitated conditions through increasing remove of mass and reducing gradients of concentration (Shahvali et al., 2000, Nienow, 2006). According to Nilsson et al. (2006), wastewater decolorization efficiency of reactive blue 4 (a blue anthraquinone dye) or reactive red 2 (a red azo dye) found in the range of 65-70% for treating a textile wastewater using the white rot fungus and for removing textile dyestuff containing wastewater by *Coriolus versicolor* in reactor at the end of three days. Kapdan and Kargi (2002) revealed out a maximum decolorization efficiency of 77% on a textile dyestuff wastewater in 24 h. In another study about biological treatment of textile effluent, Andleeb et al. (2020) studied with fungal isolate of A. terreus. They found overall color, BOD and COD removed by 84.5, 66.5 and 75.2%, respectively at 24 h period. Sedighi et al. (2009) tried to reveal out the decolorization performance of *P. chrysosporium* on textile wastewater for decolorization and COD parameters and these efficiencies were found as 87 and 42% in 4 days period. Compared with these literatures, the present study shows good reduction performance (86,28%) of *P. chrysosporium* in agitated conditions at a crude sample for decolorization.

In present study, observed high COD removal efficiency may be due to complete mineralization of highly oxidizable dyes represent in the wastewater (91,46%). This means COD value decreased to 122 ppm from 1485 ppm. This value is very suitable for dischage limits according to the water pollution control regulation in Turkey, Table 10 (Official Gazette Date/Number: 31.12.2004/25687). Similarly Pakshirajan and Kheria (2012) have reported that the COD removal may be due to biodegradation of complex organic materials such as dyes present in the wastewater. According to their study, the removal performance of *P. chrysosporium* on textile wastewater was 64 and 83% for decelorization and COD parameters at the end of 6 days. Mahmoud (2016) found an increase in COD removal percentage to 62% of some reactive dye from aqueous solution using strain of *S. cerevisiae* within 6 days.. Lin et al., (2009) has degraded synthetic dye water, with partial decolorization. In this study, there was total decolorization with 90% of COD and 83% of color reduction in 70 hour period. Faraco et al. (2009) have obtained 31% COD reduction of acid dye wastewater by white-rot fungus *P. ostreatus* treatment at one week. Compared with these three literatures, the study reveals high COD removal efficiencies (91,46%) by *P. chrysosporium* in agitated conditions.

We have obtained 94,92% TOC reduction in agitated conditions with 1:10 diluted wastewater after 24 h. Hai et al. (2005) reported TOC removal from a synthetic textile wastewater by a white rot fungus *Coriolus versicolor* was about 97% in 24 hours. Hossain et al. (2016) studied bioremediation of the Textile Wastewater using white-rot fungus *Coriolus versicolor*. At the end of the study, they revealed out TOC removal about 97% within 2 weeks. Mohamed et al. (2016) achieved nearly 75% TOC reduction of textile effluent by *Neurospora sp* at 48 hours and declared that the textile dye used as a sole carbon source.

CONCLUSION

According to the results of the study, the live pellets of the *P. chrysosporium* were found to remove more than 86% of the color of this textile wastewater at the end of the 48th hours. This time is very suitable time when comparing with the similar studies. This microbial source found to be alternative process for the treatment of textile effluent. Experiments are planned to further examine the pathways and enzymes involved in the degradation of these dyes. The dye-removal capacity was a function of exposure time and was proportional to the agitation or static media. The decolorization performance of *P. chrysosporium* remained high and this fungus showed up to 90% removal efficiency for COD and TOC parameter within two days. Our results suggest that *P. chrysosporium* pellets can play an important part to the bioremediation of industrial textile wastewater.

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